IN THE DRAWINGS:

Please replace the originally filed Figures 2A and 2B with the Figures 2A and 2B enclosed herewith.

SEQUENCE LISTING:

Please insert the sequence listing enclosed herewith after the Drawings in the above-referenced patent application.

REMARKS

Status of the Claims

Claims 1-28 are pending. Claims 1-28 are rejected. Claims 1-2, 5, 10, 15, 17-19, and 28 are amended herein. Claims 3-4, 13-14, 16, and 26-27 are canceled.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendments. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**". Reconsideration of the pending claims is respectfully requested.

Amendments to the claims

Independent claims 1 and 15 are amended to overcome prior art rejections under 35 U.S.C. 102(e) and 103(a) as listed *infra* and to further overcome the 35 U.S.C. 112, second paragraph rejections. Amendments have incorporated suggestions helpfully made by the Examiner. No new matter has been added.

Informalities

a) All parts of the collective Figure 1 were not identified in the specification. Applicants have amended the Brief Description of the Drawings for Figure 1 to identify which components of the figure are Figure 1A and Figure 1B.

b) Citations at page 5, lines 6 and 8 are incomplete. Applicants submit that the Gilardi *et al.* and the Marvin and Hellinga references are identified by number within the sentence on page 5, lines 5-10 and fully disclosed in the references cited at the end of the specification.

c) Figures 2A/2B do not have sequence identifiers as required by 37 CFR 1.821-1.825. Applicants have amended Figures 2A/2B to identify the sequences disclosed therein. Applicants further submit a copy of the Figures 2A/2B showing the corrections

in red and an amended Figure 2A/2B incorporating the amendments. No new matter is contained within the amended Figures 2A/2B.

d) In response to a Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures included with the instant Office Action Applicants submit herewith a paper copy of the Sequence Listing, a Computer Readable Form of the Sequence listing filed herewith, a Sequence Compliance Statement and a copy of the Notice to Comply with Requirements under 37 CFR 1.821-1.825 and an amendment to insert the sequence listing into the instant application. No new matter is included in the Sequence Listing.

Objection to the Specification

The specification was objected to for failing to provide proper antecedent basis for use of the negative limitation in claim 10 that the aptamer is not a biopolymer. Applicants have amended claim 10 to delete the limitation. As defined within the specification and as is known in the art an aptamer is a nucleic acid sequence which can be relatively small compared to potential target molecules. **Gold** *et al.* in U.S. 5,270,163, which teaches the SELEX method and is cited in the Description of the Related Art of the instant application,

discloses that aptamer target molecules include natural and synthetic polymers, including proteins, polysaccharides, glycoproteins, hormones, receptors and cell surfaces. These are what constitute biopolymers. Aptamers are not their target molecules.

The 35 U.S.C. §112, second paragraph rejections

Claims 1-28 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection.

a) The claims were considered confusing as to whether the method detects the presence of a ligand bound to a signaling aptamer by detecting a change in signal from a reporter molecule after binding or for transducing a change in the signal from a signaling aptamer comprising the reporter molecule. Applicants have amended claims 1 and 15 to recite a method of transducing a conformational change of a signaling aptamer upon binding a ligand to a detectable differential signal/optical signal generated by a reporter molecule appended to the signaling aptamer prior to binding the ligand.

b) In the preamble and method steps "reporter molecule/fluorescent dye" were considered to lack antecedent basis

in that there is no relationship to the signaling aptamer indicated. Applicants have amended claims 1 and 15 to recite "a reporter molecule/fluorescent dye" appended to the signaling aptamer prior to binding the ligand. Claim 15 has been further amended to incorporate the limitation of claim 18 reciting that the fluorescent dye is appended so as not to interfere with a ligand-binding site on the signaling aptamer.

c) The use of "wherein" in the contacting step in claims 1 and 15 was considered grammatically incorrect. As helpfully suggested by the Examiner, Applicants have amended claims 1 and 15 to recite the phrase --under conditions whereby--.

d) In the "detecting" step "thereby transducing the conformational change" does not flow from "detecting". Applicants have amended claims 1 and 15 to recite the phrase --the differential signal generated by the reporter molecule transduced by the conformational change --.

e) In claims 4 and 5 the introduced "nucleic acid binding species (aptamer)" was considered confusing because it describes a ligand. Applicants have deleted claim 4 and amended claim 5 to recite only aptamer. Applicants have defined "aptamer" in the specification.

f) In claims 4 and 5 the introduced "nucleic acid binding species (aptamer)" was considered confusing because it lacks antecedent basis in claims 1 and 4, respectively. Applicants have deleted claim 4 and amended claim 5 to recite that the reporter molecule is appended to an aptamer by covalent or non-covalent coupling thereby forming the signaling aptamer. Applicants have defined "aptamer" and "signaling aptamer" in the specification.

g) In claim 10, the phrase "wherein the aptamer...is not a biopolymer" was considered confusing. Applicants have amended claim 10 as suggested by the Examiner and additionally, claim 10 was amended to delete "protein".

h) Claim 28 was considered confusing as improperly depending from claim 15. As helpfully suggested by the Examiner, Applicants have amended claim 28 to recite –The method of claim 15 wherein the ligand is quantitated by the step comprising...--.

i) Claim 28 lacks proper antecedent basis in claim 15 for "the increase in the optical signal". Applicants have amended claim 28 to recite a step of correlating the optical signal generated upon the signaling aptamer binding the ligand with the quantity of ligand bound to the signaling aptamer.

Accordingly, in view of the claim amendments presented *supra*, Applicants respectfully request that the rejection of claims 1-28 under 35 U.S.C. § 112, second paragraph, be withdrawn.

The 35 U.S.C. §102(b) rejections

Claims 1-13, 15-17, 19, 23, and 25-26 are rejected under 35 U.S.C. §102(b) as being anticipated by **Pitner** et al. (U.S. 5,650,275). Claims 1-13, 15-17, 19, 23, 25-26, and 28 are rejected under 35 U.S.C. §102(b) as being anticipated by **Royer** (U.S. 5,445,935). Claims 1-4, 7-17, 19, 23, and 25-27 are rejected under 35 U.S.C. §102(e) as being anticipated by **Gold** et al. (U.S. 6,242,246). Applicants respectfully traverse these rejections.

Regarding claims 1 and 15, the Examiner states that **Pitner** *et al.*, **Royer** and **Gold** *et al.* teach the claimed method of detecting a differential signal of a signaling aptamer (detectably labeled nucleic acid ligand/fluorescent labeled polynucleotide/ detectably labeled nucleic acid ligand) upon binding a ligand (target molecule/macromolecule/target molecule), the differential signal generated by a reporter molecule (spectroscopically detectably labeled nucleic acid ligand/fluorescent label/fluorescent label) with the ligand where the former binds (complexes with/binds) with the

latter and detecting the differential signal generated by the reporter molecule (spectroscopically detectable label/fluorescent label/fluorescent label measured before and after binding) (**Pitner** *et al.*: col. 13-14, claim 1; **Royer**: col. 17, claim 1; **Gold** *et al.*: Abstract, lines 2-14, col. 15, lines 49-53, col. 16, lines 54-57). Additionally, the recitation in the instant claims of "transducing the conformational change of a signaling aptamer upon binding a ligand to a differential signal" is inherent in the claim 1 methods of **Pitner** *et al.*, **Royer** and **Gold** *et al.* because it was known in the prior art (see Description of the Related Art in the instant application) that aptamers undergo an induced fit conformational change in the presence of their cognate ligands (**Pitner** *et al.*: col. 2, lines 55-59; **Royer**: sic; **Gold** *et al.*: col. 15, lines 49-52, col. 16, lines 54-56).

Pitner *et al.* teach a method of detecting a target compound in a sample by measuring the fluorescent polarization or fluorescent anisotropy of a fluorescently labeled receptor molecule and subsequently measuring these values when the receptor molecule is placed in solution with the target compound. Polarization and anisotropic values depend on the degree of depolarization of the emitted light from the excited molecule relative to the parallel and perpendicular planes of the polarized excitation light. These values

depend upon solvent diffusion and tumbling motion of the fluorescent molecule. If a fluorescently labeled receptor molecule binds with the target compound, its size is effectively increased and the tumbling slows changing the polarization. Thus, **Pitner** *et al.* requires a two-step comparative measurement as recited in their claim 1.

Applicant has amended claims 1 and 15 to recite monitoring for a differential optical signal that is a change in fluorescence intensity or a change in colorimetric intensity after contacting the signaling aptamer with the ligand (target). Fluorescence intensity and fluorescence polarization measurements are not the same. Although binding a target molecule to a fluorescently labeled receptor molecule causes the fluorphor to emit differently fluorescently polarized light as described *supra*, the instant fluorphor in the ligand, upon binding the target molecule, may or may not simply fluoresce relative to its unbound state. **Pitner** *et al.* state that due to the small size of the labeled receptor molecules, any target molecule will significantly increase the volume and weight of the labeled receptor and therefore permit detection of the change in fluorescence polarization (col. 2, lines 59-64).

Additionally, the fluorphor in **Pitner** *et al.* may be attached practically anywhere in the receptor molecule (col. 6, lines 47-60) and a change of fluorescent polarization will occur upon binding the target. In the instant application, positioning of the fluorophore relative to the binding site(s) of the aptamer is crucial. Although a fluorophore may not interfere with the binding site, its position within the aptamer determines whether or not it fluoresces when the signaling aptamer undergoes a conformational change upon binding the ligand. Contrary to the Examiner's assertion, an appended dye in the instant invention does not necessarily undergo a ligand-dependent change in its local environment sufficient enough to induce simple fluorescence. Furthermore, **Pitner** *et al.* require a two-step comparative measurement as recited in their claim 1. Applicant's method only requires monitoring for a differential fluorescence intensity or colorimetric signal after contacting the signaling aptamer with the ligand (target).

Royer teaches a method of quantitation of a macromolecule in solution by measuring the changes in fluorescence polarization upon the association of the macromolecule with an oligonucleotide labeled with a fluorophore covalently coupled to the oligonucleotide (see abstract and claim 1). This is essentially the

method of **Pitner** *et al.* as it applies to Applicant's invention and, thus, Applicant's arguments and remarks are as made *supra*.

Gold *et al.* disclose a nucleic acid ligand biochip having a solid support to which one or more specific nucleic acid ligands is attached in a spatially defined manner. The nucleic acid ligands are contacted by a target molecules and if the target is bound by the nucleic acid ligand or receptor a detectable change occurs which is a change in fluorescence or a change in a physical property, e.g., electrical conductivity or refractive index (see Abstract). **Gold** *et al.* further disclose that a fluorophore such as fluorescein or Texas Red may be attached to the ligand on the biochip and binding of the target can be determined by measuring a change in fluorescence intensity, fluorescence polarization, fluorescence anisotropy and fluorescence lifetime (col. 15, lines 42-65). Applicant has canceled claims 13-14 and 26-27 and amended claims 1 and 15 to recite the signaling aptamer as being in solution.

Regarding dependent claims 2-13, 16-17, 19, 23, and 25-28, Applicants have canceled claims 3-4, 13-14, 16, and 26-27. The remaining dependent claims are drawn to methods of appending the reporter molecule/fluorescent dye to the aptamer, the types of molecules used for the aptamers, reporter molecules and dyes,

specific types of signaling aptamers and a method of quantitation using the methods recited in independent claims 1 and/or 15. As these are dependent claims, neither **Pitner** *et al.*, **Royer** nor **Gold** *et al.* can anticipate Applicant's invention through these claims, if **Pitner** *et al.*, **Royer** nor **Gold** *et al.* do not anticipate amended independent claims 1 and 15.

For a valid §102 rejection, the prior art references must contain each element of the claimed invention. Absent teachings of using the generation of fluorescence, as measured in a single step by intensity, by a fluorphore attached to an aptamer resulting from the conformational change of the aptamer upon binding a ligand, neither **Pitner** *et al.* nor **Royer** anticipate Applicant's claimed invention. Also, absent teachings of detecting this same change of a fluorphore in solution, **Gold** *et al.* does not anticipate the instant invention. Therefore, as this reference is not valid prior art against the instant application under 35 U.S.C. §102 and in view of the preceding amendments and remarks, Applicant respectfully submits that the cited references do not anticipate claims 1-13, 15-17, 19, 23, and 25-28 under 35 U.S.C. §102. Accordingly, Applicants respectfully request that the rejection of claims 1-13, 15-17, 19, 23, and 25-28 under 35 U.S.C. §102(b) and §102(e) be withdrawn.

The 35 U.S.C. §103(b) rejections

Claims 14, 18, 20-22, 24 and 27 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Pitner** *et al.* as applied to claims 1-13, 15-17, 19, 23, 25, 26, and 28 above, and further in view of **Gold** *et al*, **Conrad** (U.S. 5,728,525) and **Szostak** *et al.* (U.S. 5,631,146). Claim 28 stands rejected under 35 U.S.C. §103(a) as being unpatentable over **Pitner** *et al.* as applied to claims 1-13, 15-17, 19, 23, 25, 26, and 28 above and further in view of **Royer**. Applicant respectfully traverses these rejections.

With regard to claims 14 and 27, Applicant has canceled these claims thereby rendering the rejection moot.

With regard to claim 18, the Examiner argues that it would have been obvious and the skilled practitioner would have been motivated at the time the claimed invention was made to label the nucleic acid ligand of **Pitner** *et al.* by replacing a nucleic acid residue with a fluorescent dye as disclosed in **Conrad** by replacing the residue during chemical or enzymatic synthesis. In considering claims 20-22 and 24, the Examiner further states a skilled practitioner would be motivated to use an anti-adenosine aptamer in the method of **Pitner** *et al.* in lieu of **Pitner's** teaching that many

aptamers are known in the art and that specific sequences can be synthesized. Further it would have been obvious to use the aptamers of the instant claims 21 and 22 in view of **Szostak** *et al.* teaching a large number of anti-adenosine aptamers having the same conserved region as the aptamer of claim 22 with the expectation that they would function in the same manner as Applicant's aptamers.

Pitner *et al.* is described *supra*. **Conrad** teaches nucleoside analogs which are fluorescent and are useful as monomers in synthesizing and labelling nucleotide sequences. These monomers can substitute for naturally occurring nucleosides in the synthesis of oligonucleotide probes (Abstract; col. 6, lines 45-51). **Szostak** *et al.* teach single-stranded DNA molecules which bind adenosine or an adenosine-5'-phosphate and methods for producing them.

Applicant argues that at best, one skilled in the art might find it obvious to try various combinations of the aptamers and fluorescent dyes culled from **Conrad** and **Szostak** *et al.*, however, to try does not render the instant invention obvious absent a suggestion that such efforts would be successful. As stated in the instant application, several anti-adenosine RNA aptamers or an anti-adenosine DNA aptamers, although homologous in structure and

function to an aptamer as taught in **Szostak** *et al.* in so far as the aptamers bind an appropriate ligand, and containing a fluorescent dye, as taught in **Pitner** *et al.* and positioned within the aptamer as in **Conrad**, do not fluoresce upon contact with the ligand (pg. 15, line 27–pg. 16, line 23). Applicant argues that any aptamer/fluorescent dye construct arrived at would not render the instant invention obvious because **Pitner** *et al.* itself does not teach nor suggest Applicant's method as recited in amended claims 1 and 15.

With regard to claim 28, the Examiner states that a skilled practitioner in the art would be motivated to combine the quantitative method of **Royer** with the method of **Pitner** *et al.* in view of the teaching of **Pitner** *et al.* that their method can be used to quantitatively determine the presence of a target molecule (ligand) and further in view of the similarity of the **Royer** and **Pitner** *et al.* methods with regard to the binding (complexing) of a macromolecule or target molecule with a fluorescently labeled polynucleotide (signaling aptamer) and the measuring of fluorescence polarization. Both **Pitner** *et al.* and **Royer** are as described *supra*.

As amended, claim 15 recites only the detectable optical signals of fluorescence intensity and colorimetric intensity. **Pitner** *et al.* and **Royer** both use fluorescence polarization as a detectable

signal. There is no suggestion in either reference to measure fluorescence intensity or colorimetric intensity. Applicant has amended claim 28 to recite a single step of correlating the optical signal upon binding of the ligand to the amount of ligand bound. Again, claim 28 recites a quantitative method dependent upon the method of amended claim 15 of transducing a conformational change of a signaling aptamer upon binding a ligand to a detectable fluorescent intensity or colorimetric intensity detectable signal of a fluorescent reporter. Thus, combining **Royer's** method with the method of **Pitner** *et al.* to quantitate a ligand does not render the instant invention obvious because **Pitner** *et al.* and **Royer,** neither singly nor in combination, render the invention as recited in amended claim 15 obvious.

In view of the above remarks, Applicants respectfully submit that obviousness can not be established by combining the teachings of the prior art absent some teaching, suggestion or motivation supporting the combination to do so. Absent a suggestion or teaching in **Pitner** *et al.* of using fluorescence intensity or colorimetric intensity as a detectable signal, Applicants' invention as recited in amended claim 1 is not rendered obvious by combining any of the particular signaling aptamers or aptamers or methods of

quantitation of **Conrad, Szostak** *et al.* or **Royer** with **Pitner** *et al.* Thus, the invention as a whole was not obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 14, 18, 20-22, 24 and 27-28 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Office Action mailed March 29, 2002. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution. Applicants believe that no fees are due, however, should this be in error, please debit Deposit Account No. 07-1185 on which the undersigned is allowed to draw.

Respectfully submitted,

Date: August 23, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning on line 1 of page 9 has been amended as follows:

Figure 1 shows the three-dimensional models of anti-adenosine aptamers derived from NMR analysis.[11,12] Some of the sites chosen for dye incorporation into either RNA, ATP-R-Ac13 (blue) (**Figure 1A**), or DNA, DFL7-8 (orange) (**Figure 1B**), aptamers are shown in yellow. Bound adenosines are shown in purple.

Paragraph beginning on line 6 of page 9 has been amended as follows:

Figure 2 shows the sites of dye incorporation into RNA (SEQ ID NO: 1) and DNA aptamers (SEQ ID NO: 2). In **Figure 2A** in the RNA aptamers acridine is incorporated in place of residue 13 (ATP-R-Ac13). Fluorescein is incorporated at the 5' end (ATP-R-F1), at the 5' end with a heptaadenyl linker (SEQ ID NO: 3) (ATP-R-F2), and in place of residue 13 (ATP-R-F13). In **Figure 2B** in the DNA aptamers. fluorescein was incorporated at the 5' end (DFL0), in place

of residue 7 (DFL7), and in between residues 7 and 8 (DFL7-8). Residues are numbered from the 5' end on the secondary structures.

IN THE CLAIMS:

Please amend claim 1 as follows:

1. (amended) A method of transducing the conformational change of a signaling aptamer that occurs upon the signaling aptamer binding a ligand to a detectable differential signal generated by a reporter molecule that is appended to the signaling aptamer prior to binding the ligand comprising the steps of:

contacting the signaling aptamer in solution with the ligand ~~wherein~~ under conditions whereby the signaling aptamer binds the ligand; and

detecting the differential signal generated by the reporter molecule ~~resulting from~~ transduced by the conformational change ~~of~~ in the signaling aptamer upon binding the ligand ~~thereby transducing the conformational change~~ wherein the differential signal is an optical signal expressed as fluorescence intensity or colorimetric intensity.

Please amend claim 2 as follows:

2. (amended) The method of claim 1, wherein the differential signal <u>further</u> comprises ~~an optical signal,~~ an electrochemical signal or an enzymatic signal.

Please amend claim 5 as follows:

5. (amended) The method of claim ~~4~~ <u>1</u>, wherein the reporter molecule is appended to <u>an</u> ~~the nucleic acid binding species (aptamer)~~ by covalent coupling or non-covalent coupling <u>thereby forming the signaling aptamer</u>.

Please amend claim 10 as follows:

10. (amended) The method of claim ~~4~~ <u>5</u>, wherein the aptamer is selected from the group consisting of RNA, DNA, modified RNA and modified DNA, ~~and wherein the aptamer is not a protein or a biopolymer~~.

Please amend claim 15 as follows:

15. (amended) A method of transducing the conformational change of a signaling aptamer <u>that occurs</u> upon <u>the signaling aptamer</u> binding a ligand to a~~n~~ <u>detectable</u> optical signal generated by a fluorescent dye <u>that is appended to the signaling</u>

aptamer at a site that does not interfere with a ligand-binding site of the signaling aptamer prior to binding the ligand comprising the steps:

contacting the signaling aptamer in solution with the ligand ~~wherein~~ under conditions whereby the signaling aptamer binds the ligand; and

detecting the optical signal generated by the fluorescent dye ~~resulting from~~ transduced by the conformational change ~~of~~ in the signaling aptamer upon binding the ligand, ~~thereby transducing the conformational change~~ wherein the optical signal is expressed as fluorescence intensity or colorimetric intensity.

Please amend claim 17 as follows:

17. (amended) The method of claim 15, wherein the ~~signaling aptamer comprises a~~ fluorescent dye is appended to ~~a~~ an ~~nucleic acid binding species~~ ~~(~~aptamer~~)~~ by covalent coupling ~~of the fluorescent dye to the~~ thereby forming the signaling aptamer.

Please amend claim 18 as follows:

18. (amended) The method of claim 17, wherein the fluorescent dye replaces a nucleic acid residue in the aptamer or is inserted between two nucleic acid residues in the aptamer; ~~wherein~~

~~the placement does not interfere with the ligand-binding site of the aptamer~~.

Please amend claim 19 as follows:

19. (amended) The method of claim ~~1-7~~ 15, wherein the fluorescent dye is fluorescein or acridine.

Please amend claim 28 as follows:

28. (amended) ~~A~~ The method ~~for quantitating the ligand~~ of claim 15, wherein the ligand is quantitated by ~~comprising~~ the steps ~~of~~ comprising:

contacting the signaling aptamer of claim 15 with the ligand wherein the signaling aptamer binds the ligand; and

~~measuring the increase in the optical signal of claim 15 resulting from the signaling aptamer binding the ligand; wherein the increase in the optical signal positively correlates with the quantity of ligand bound to the signaling aptamer~~

correlating the optical signal generated upon the signaling aptamer binding the ligand with the quantity of ligand bound to the signaling aptamer.

Please cancel claims 3-4, 13-14, 16, and 26-27.